Major Effect of Pyrrolic N-Benzylation in Norbinaltorphimine, the Selective κ-Opioid Receptor Antagonist

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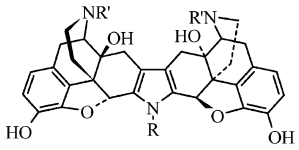
Indolic N-benzylation of naltrindole reportedly extends the duration of δ-opioid receptor (DOR) antagonism. Similar modification of the κ-opioid receptor (KOR) antagonist norBNI (**1a**) and its 17,17′-diNMe analogue (**1d**), a low potency μ-opioid receptor (MOR) partial agonist, was found to affect predominantly their MOR activity. When administered systemically in mouse antinociceptive assays, *N*-benzyl-norBNI (**1b**) had only MOR agonist activity of relatively short duration whereas on central administration it had only a KOR-antagonist action of extremely long duration.

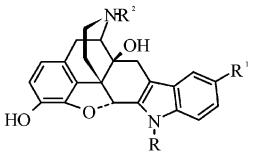
Introduction

For the past 30 years there has been considerable interest in the development of nonpeptidic ligands for κ (KOR) and δ (DOR) opioid receptors. Discovery of antagonists with selectivity for KOR[1−3] and DOR[1,4] has enabled the function of these receptor systems to be explored. The prototype KOR antagonist norBNI (**1a**) was designed on the message−address principle in which the address component which confers KOR selectivity is the basic nitrogen of the second *N*-cyclopropylmethyl (CPM) group.[5] The follow-up to norBNI was GNTI (**2b**) in which the guanidino KOR address component was introduced into the selective DOR-antagonist, naltrindole (NTI, **2a**).[6] The effect of benzyl substitution at the indole N-atom in NTI to give BNTI (**2c**) was to bring selectivity for the putative DOR_2-subtype in an antagonist of substantially longer duration than NTI.[7] It was thus of interest to investigate the effect of equivalent N-benzylation of norBNI (**1a**) and its 17,17′-diNMe analogue (**1d**). Herein we report the results of the in vitro (binding and [^{35}S]GTPγS) pharmacological evaluation of **1b** and analogues, plus the further evaluation of **1b** in vivo.



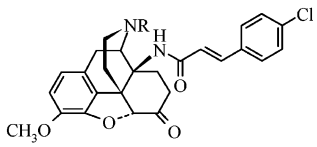
1

a: R = H, R′ = $CH_2cC_3H_5$
b: R = $CH_2C_6H_5$, R′ = $CH_2cC_3H_5$
c: R = CH_3, R′ = $CH_2cC_3H_5$
d: R = H, R′ = CH_3
e: R = $CH_2C_6H_5$, R′ = CH_3



2

a: R = R^1 = H, R^2 = $CH_2cC_3H_5$ NTI
b: R = H, R^1 = NHC(=NH)NH_2, R^2 = $CH_2cC_3H_5$ GNTI
c: R = $CH_2C_6H_5$, R^1 = H, R^2 = $CH_2cC_3H_5$ BNTI
d: R = R^1 = H, R^2 = CH_3 OMI
e: R = H, R^1 = NHC(=NH)NH_2, R^2 = CH_3



3

a: R = $CH_2cC_3H_5$
b: R = CH_3

Chemistry. Two complimentary methods were utilized for the synthesis of BnorBNI (**1b**). In the first, the reported method for the synthesis of BNI (**1c**) from naltrexone (**4**) and *N*-methylhydrazine sulfate[8] was modified by the use of *N*-benzylhydrazine sulfate and extending the reaction time to one week, then at elevated temperature for 2 days. Unfortunately, under these conditions only 1% of **1b** could be isolated with the major identifiable material appearing to be hydrazone (**5**) in 20% yield (Scheme 1). The alternative method, direct benzylation of **1a** (prepared from naltrexone in 62% yield)[9] using excess sodium hydride and benzyl-bromide, yielded a mixture of tri- and pentabenzyl-substituted norBNI, that was hydrolyzed immediately in hydrochloric acid/methanol to yield **1b** in 63% yield (39% from naltrexone). Similar treatment of **1d**, prepared from oxymorphone (**4b**), with benzyl bromide yielded the tribenzyl-substituted compound that was hydrolyzed to **1e** with HBr/MeOH.

Results and Discussion

Affinities of the compounds for opioid receptors were measured using radioligand binding assays in membranes from C6μ, C6δ, and CHOκ cells and competition for the nonselective antagonist [^{3}H]diprenorphine as previously described.[10] BnorBNI (**1b**) had subnanomolar affinity for KOR with modest selectivity over MOR and DOR (Table 1). When compared with norBNI (**1a**), the prototypic KOR-antagonist **1b** was as, or slightly more, selective under these conditions. The modest selectivity observed for these compounds was not surprising since Takemori et al.[3] showed that binaltorphimine (BNI, **1c**) had very little KOR selectivity in binding assays. The 17-NMe analogues (**1d**, **1e**) had at least an order of

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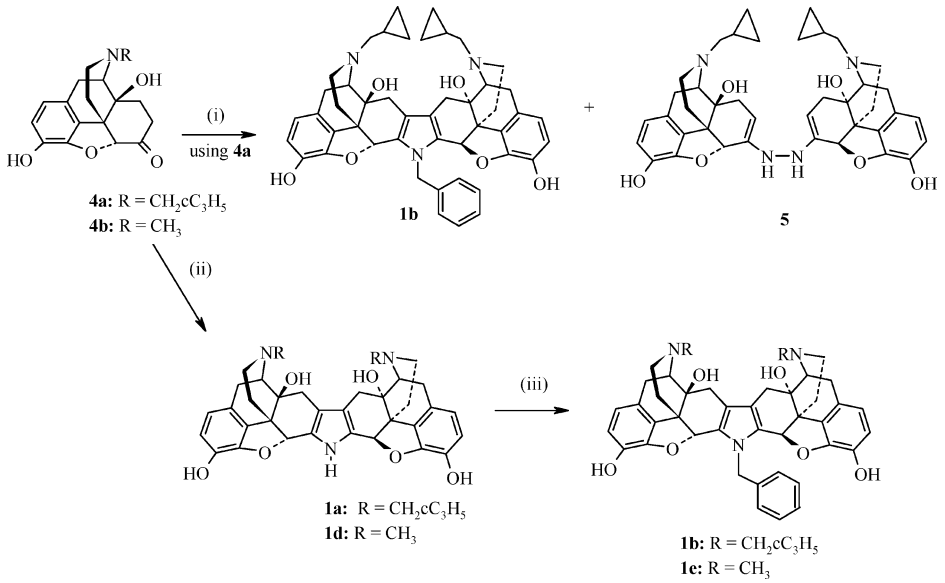
Scheme 1[a]



[a] Reagents and conditions: (i) $BnHNNH_2.H_2SO_4$, AcOH (ii) H_2NNH_2, DMF, 100 °C then $MeSO_3H$, DMSO, 130 °C (iii) NaH, 18-crown-6, BnBr then methanol, 12 N HCl, 90 °C (for **1b**), HBr, r.t. (for **1e**).

Table 1. Binding Affinities for Ligand Binding to Opioid Receptors[a]

	K_i (nM) ± SEM[b]				
compound	MOR	DOR	KOR	MOR/KOR	DOR/KOR
1a, norBNI	1.20 ± 0.2	5.8 ± 0.645	0.4 ± 0.06	3	15
1b, BnorBNI	10.0 ±2.5	8.6 ± 0.7	0.7 ± 0.1	14	12
1d	63.6 ± 39.1	98.2 ± 25.3	7.7 ± 1.0	8	13
1e	6.4 ± 1.1	207 ± 96	24.9 ± 5.6	0.3	8

[a] Rat MOR or DOR receptors in C6 cells and human KOR receptors in CHO cells. [b] Values are the mean of three experiments, each performed in duplicate. Experiments were performed as described in ref 10 using [^{3}H]diprenorphine.

Table 2. Agonist Effects of Ligands at Opioid Receptors Measured by the [^{35}S]GTPγS Binding Assay[g]

	EC_{50}/nM, % stim				
compd	MOR[a]		DOR[b]	KOR[c]	
1a	-[e]		-[e]	-[e]	
1b	187[d]	38	-[e]	1906[f]	29
1d	1388 ± 370	66	-[e]	-[e]	
1e	526 ± 279	54	-[e]	-[e]	

[a] Compared to the full agonist DAMGO. [b] Compared to the full agonist SNC80. [c] Compared to the full agonist U69593. [d] 95% CI 42-844nM. [e] No stimulation up to 10 000 nM. [f] 95% CI 269-13490 nM. [g] Experiments were performed using membranes from Rat MOR or DOR receptors in C6 cells and human KOR receptors in CHO cells as described in ref 11. Values are from three separate experiments.

magnitude lower affinity than **1a** and **1b** at each receptor. The exception was for **1e** at the MOR where affinity was similar to **1b**, resulting in **1e** displaying modest selectivity for MOR.

In the [^{35}S]GTPγS functional assay[10,11] in C6μ, C6δ, and CHOκ cells Bnor BNI (**1b**) showed partial MOR and KOR agonism, reaching approximately 40% and 30% respectively of maximal effect compared with the appropriate full agonists at each receptor (Table 2). The MOR partial agonist effect of BnorBNI, though of modest potency, was ten-times higher than its potency as a KOR-partial agonist. In contrast, there are no unequivocal reports in the literature that **1a** or **1c** have opioid agonist actions in vitro or in vivo, with evidence only of very limited agonism in isolated tissue assays.[12] However, Takemori et al.[3] reported that 24.5 μmol/kg **1a** sc enhanced the potency of the selective peptidic MOR agonist DAMGO 3-fold in the acetic acid induced stretching assay in mice (AS).[3] As expected from standard opioid SAR regarding 17-NMe and 17-NCPM substituents, **1d** and **1e** had higher efficacy at MOR than norBNI (**1a**) and BnorBNI (**1b**) but were substantially less potent. However, the additional benzyl group in **1e** increased MOR potency rather than efficacy compared to **1d**, which is in contrast to the **1a**/**1b** comparison where the additional benzyl group increased MOR efficacy. The activity of **1d** in the [^{35}S]GTPγS assay, low potency MOR partial agonist, is in agreement with that of Portoghese's group who found similar activity in the guinea pig ileum assay.[9]

The antagonist potency of BnorBNI (**1b**) determined against the selective KOR agonist U69593 (K_e = 0.26 nM) was 50-fold greater than its potency determined against the selective DOR agonist SNC80 and nearly 100-fold greater than its potency against the MOR agonist DAMGO (Table 3). Once again these data for BnorBNI (**1b**) compare favorably with those obtained for norBNI (**1a**) (50- and 22- fold selective for KOR over DOR and MOR respectively) under the same assay conditions. Thus, the selectivity of the KOR antagonist action of both norBNI and BnorBNI is substantially greater in this functional assay than their KOR-selectivity in binding assays. The 17-NMe analogues (**1d**, **1e**) were very much less potent KOR antagonists than norBNI and BnorBNI to the extent of 2−3 orders of magnitude (Table 3).

It is of interest to compare the binding and in vitro effects of 17-N-Me versus 17-N-CPM substitution in the

Table 3. Antagonist Effects of Ligands at Opioid Receptors Measured by the [^{35}S]GTPγS Binding Assay[b]

compd	K_e (± SEM)/nM		
	MOR[a]	DOR[a]	KOR[a]
1a	2.38 ± 0.58	5.17 ± 0.73	0.11 ± 0.01
1b	25.5 ± 2.3	13.3 ± 4.5	0.26 ± 0.085
1d	NT	NT	27.0 ± 4.3
1e	NT	NT	311 ± 30

[a] K_e values were determined from dose−response curves for DAMGO (MOR), SNC80 (DOR), and U69593 (KOR) in the presence or absence of test ligand according to the formula: K_e = [antagonist]/(dose-ratio − 1). [b] Experiments were performed using membranes from Rat MOR or DOR receptors in C6 cells and human KOR receptors in CHO cells as described in ref 11. Values are from 3 separate experiments.

current series with equivalent substitutions in series of selective OR ligands having indolomorphinan structures. The 17-NMe analogue (**2e**) of GNTI (**2b**) in binding assays had substantially lower KOR affinity than GNTI but greater KOR selectivity as a result of even lower MOR and DOR affinity.[13] This was also true of OMI (**2d**) in comparison to NTI.[14,15] In the present series the loss of affinity in substituting 17-NCPM by NMe is similar for KOR and DOR so that there is no gain of KOR over DOR selectivity. There was a small gain of KOR over MOR selectivity from norBNI (**1a**) to **1d**, but the reverse was true when the pyrrolic *N*-benzyl group was introduced (**1e** versus **1b**).

The effects on the in vitro OR functional profiles of GNTI (**2b**) and NTI (**2a**) when the 17-NCPM group is exchanged for NMe are quite different. The analogue (**2e**) of GNTI remains a KOR antagonist but with very much lower potency and selectivity than GNTI; there was no evidence of agonist activity in isolated tissue preparations.[13] By contrast, the selective, potent DOR antagonist activity of NTI (**2a**) was totally lost in OMI (**2d**) but the latter had substantial DOR partial agonist activity.[15] In the present series the effects of 17-NMe for 17-NCPM substitution in norBNI (**1d** cf. **1a**) was reduction of KOR antagonist potency as in NTI but of greater significance was the appearance of a MOR partial agonist effect of substantial efficacy though of low potency. The latter was increased by introduction of the pyrrolic *N*-benzyl group (in **1e**).

The action of BnorBNI (**1b**) at opioid receptors was further investigated in vivo in the mouse tail withdrawal assay for antinociception (TW), in which the water temperature was 50 °C (Table 4).[16] When **1b** (10 mg/kg) was administered subcutaneously (sc) 1 h or 3 h before U69593 (sc), the dose−response curve of the agonist was shifted 2.6-fold to lower doses, suggesting an additive antinociceptive effect of **1b**. By 24 h the effect was no longer significant and at no time point (up to 48 h) was an antagonist effect detected, or at 24 h with 32 mg/kg **1b**. In contrast, norBNI (**1a**: 32 mg/kg sc, 24 h pretreatment) gave a 4-fold rightward shift of the dose−response curve for U69593.

Administration (sc) of BnorBNI (**1b**) alone in TW gave a substantial antinociceptive effect measured 30 min or 1 h after administration, but there was no significant effect by 3 h. The antinociceptive effect of BnorBNI (**1b**) was not inhibited by norBNI (**1a**) (data not shown) but was antagonized by the selective MOR antagonist methocinnamox (M-CAM)[16] (Figure 1), indicating it was mediated by an agonist effect at MOR. In contrast

Table 4. Effect of Ligands on the Dose−Effect Curve for U69593 Administered sc in the Warm Water Tail Withdrawal Assay (TW) after Systemic (sc) or Central (icv) Administration[a]

route of admin	treatment conditions applied to U69593	EC_{50}, mg/kg	fold shift	shift significance p value[c]
	+vehicle at −1 h[b]	5.2	—	—
sc	+10 mg/kg **1b** at −1 h	2.0	−2.56	0.0002
sc	+10 mg/kg **1b** at −3 h	2.1	−2.55	0.0145
sc	+10 mg/kg **1b** at −18 h	9.0	1.73	n.s.
sc	+10 mg/kg **1b** at −24 h	4.4	−1.17	n.s.
sc	+32 mg/kg **1b** at −24 h	5.7	1.09	n.s.
sc	+10 mg/kg **1b** at −48 h	9.9	1.90	n.s.
sc	+32 mg/kg **1a** at −24 h	22.7	4.12	0.0003
icv	+10 nmol **1b** at −1 h	49.1	9.41	<0.0001
icv	+10 nmol **1b** at −24 h	26.3	5.04	0.0003
icv	+10 nmol **1b** at −48 h	74.2	14.22	<0.0001
icv	+10 nmol **1b** at −168 h	37.0	7.09	<0.0001
icv	+10 nmol **1a** at −24 h	31.8	6.10	<0.0001

[a] Assays were performed as previously described in ref 12. Negative shift values reflect a leftward shift; positive values reflect a rightward shift. Significance of the shifts in agonist effect was determined by 2-way ANOVA. [b] Vehicle is 10% DMSO in normal saline. [c] n.s. = not significant.

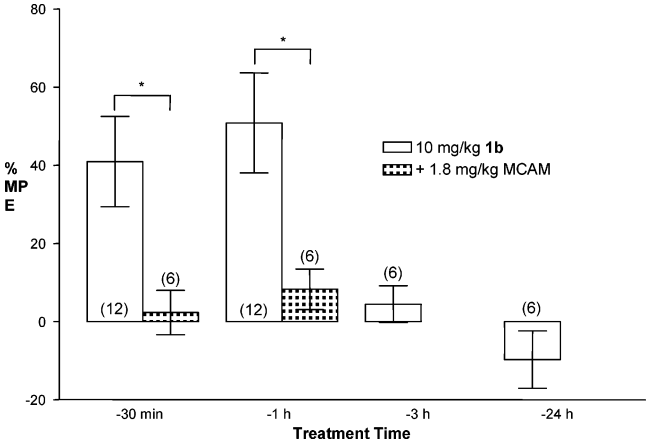


Figure 1. Agonist activity of BnorBNI (**1b**) in the tail withdrawal assay.

norBNI (**1a**) has no antinociceptive activity in the AS antinociceptive assay when administered sc but is an effective and selective KOR antagonist.[17]

When administered icv, BnorBNI (**1b**) had no agonist effect in the TW test but was an effective and selective KOR antagonist lasting at least 168 h, with the peak effect around 48 h (Table 4). The antagonist selectivity of BnorBNI (**1b**) (icv) was assessed after 1 h pretreatment by comparison of its ability to antagonize U69593 (KOR) compared with its antagonism of SNC80 (DOR) and morphine (MOR). In the case of SNC80 the water temperature in TW was set at 48 °C since DOR agonists have very little antinociceptive effect at higher temperatures. Compared with the 9-fold shift in the U69593 dose−effect curve there was no inhibition of the agonist effects of SNC80 and morphine in the presence of 10 nmoles BnorBNI (**1b**) (data not shown). This confirms that BnorBNI (**1b**) is a substantially selective KOR antagonist under these conditions, in accord with its selectivity as a KOR antagonist in the [^{35}S]GTPγS assay. At 24 h pretreatment the shift in the U69593 dose−response curve produced by 10 nmol of BnorBNI (**1b**) icv was similar to that of 10 nmol of norBNI (**1a**) (Table 4). The lack of agonist action on direct central administration of BnorBNI (**1b**) is surprising, but there

are other examples of opioid ligands with agonist and antagonist effects that exhibit agonism only when administered systemically. The 14-cinnamoylaminocodeinone (**3b**) when administered sc showed very potent and efficacious MOR-mediated antinociceptive effects in TW with no evidence of delayed antagonism.[18] In contrast on icv administration this same compound was without antinociceptive effects, but was a potent, delayed morphine antagonist.[19]

In conclusion, the effect of introducing a benzyl substituent to the pyrrole-N of norBNI (**1a**) and its 17-NMe analogue (**1d**) was predominantly on their MOR pharmacology. In the former case, the effect was to increase MOR efficacy, but not potency, while in the latter, MOR potency was increased but with no effect on efficacy. Unlike norBNI (**1a**), BnorBNI (**1b**) acts as a partial MOR agonist in vitro that shows an antinociceptive effect of relatively short duration when administered systemically. However, when administered centrally the compound lacks this effect and instead is a selective KOR antagonist of very long duration, comparable to norBNI (**1a**).

Experimental Section

Chemistry. Reagents and solvents were purchased from Aldrich or Lancaster. Melting points were recorded on a Gallenkamp MFB-595 melting point apparatus and are uncorrected. High and low resolution fast atom bombardment (FAB) mass spectra were recorded on a Fisons VG AutoSpec Q instrument, with a matrix of *m*-nitrobenzyl alcohol. Microanalyses were performed with a Perkin-Elmer 240C analyzer.

17,17′-Bis(cyclopropylmethyl)-6,6′,7,7′-tetrahydro-4,5:4′,5′-diepoxy-6,6′-(benzylimino)[7,7′-bimorphinan]-3,3′,14,14′-tetrol (1b). To a solution of norBNI[2] (0.30 g: 0.45 mmol) in dry THF under a N_2 atmosphere were added NaH (0.18 g: 4.52 mmol) and 18-crown-6 (30 mg: 0.11 mmol). This mixture was stirred for 20 min at r.t. before adding BnBr (0.16 mL: 1.36 mmol) and stirring continued for a further 43 h. The reaction was quenched by the addition of H_2O, the organic layer collected and dried ($MgSO_4$), and solvent removed in vacuo. The crude oil was purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH 400:10:1 to 290:10:1) to yield a mixture of tri- and pentabenzyl-substituted norBNI (0.33 g). This mixture was dissolved in MeOH/cHCl (20 mL, 1:1) and heated to 90 °C for 40 h. Cooling, basification (aqueous ammonia), and removal of the precipitate by filtration was followed by evaporation of the filtrate to dryness. Column chromatography (CH_2Cl_2:MeOH:NH_4OH 200:10:1) yielded BnorBNI as an off-white solid (0.21 g: 63%), MS (FAB): $m/z = 752$ $(M + H)^+$, HRMS (FAB) m/z 752.3715 $(M + H)^+$, $C_{47}H_{49}N_3O_6$ requires 751.3620, R_f (CH_2Cl_2/MeOH/NH_4OH:110/10/1) = 0.76, mp > 240°C, Anal. ($C_{47}H_{49}N_3O_6$:2HCl:5H_2O) C, H, N.

17,17′-Dimethyl-6,6′,7,7′-tetrahydro-4,5:4′,5′-diepoxy-6,6′-(benzylimino)[7,7′-bimorphinan]-3,3′,14,14′-tetrol (1e). 17,17′-Dimethyl-6,6′,7,7′-tetrahydro-4,5:4′,5′-diepoxy-6,6′-(imino)-[7,7′-bimorphinan]-3,3′,14,14′-tetrol (**1d**)[9] was treated as for **1b** above except MeOH/HBr replaces MeOH/HCl for the O-debenzylation. Yield 80%, MS (FAB): $m/z = 672$ $(M + H)^+$, HRMS (FAB) m/z 672.3063 $(M + H)^+$; $C_{41}H_{41}N_3O_6$ requires 671.2995, R_f (CH_2Cl_2/MeOH/NH_4OH:110/10/1) 0.27 mp > 240 °C, Anal. ($C_{41}H_{41}N_3O_6$:2HCl:4H_2O) C, H, N.

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Supporting Information Available: Full spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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